Chapter 12

Summary and conclusions
Role of arginase and L-arginine homeostasis in allergen-induced nitric oxide deficiency and airway hyperresponsiveness after the early asthmatic reaction

Nitric oxide (NO) is an important endogenous bronchodilator, which is produced by NO synthase (NOS) [1,2]. NOS hydrolyzes L-arginine into NO and L-citrulline, using oxygen and NADPH as cosubstrates [3]. Three NOS isoforms are known: neuronal (nNOS or NOS I) and endothelial NOS (eNOS or NOS III), which are constitutively expressed in the airways, and inducible NOS (iNOS or NOS III), which is induced by proinflammatory cytokines during airway inflammation [1,2]. The constitutive NOS (cNOS) isoforms are mainly expressed in inhibitory nonadrenergic noncholinergic (iNANC) neurons (nNOS), endothelium (eNOS) and epithelium (nNOS and eNOS), while iNOS is predominantly expressed in macrophages and epithelial cells [1,2]. While cNOS produces low levels of NO in a Ca²⁺-dependent way, iNOS produces relatively high concentrations of NO independent of Ca²⁺ [1,2]. The relatively low concentrations of cNOS-derived NO are importantly involved in airway smooth muscle relaxation, while much higher concentrations of iNOS-derived NO also contribute to airway inflammation, particularly by formation of significant amounts of the highly reactive nitrogen species peroxynitrite, the reaction product of NO and superoxide anion [1,2].

Both in animal models of allergic asthma [4-8] and in asthmatic patients [9,10] it has been shown that a deficiency of bronchodilating NO contributes to airway hyperresponsiveness (AHR). In a guinea pig model of allergic asthma, using perfused tracheal preparations ex vivo, we have previously demonstrated that a reduced availability of L-arginine to cNOS underlies the observed deficiency of agonist-induced cNOS-derived NO and AHR after the early asthmatic reaction (EAR) [11].

Using the same model, we have investigated the mechanisms underlying the observed L-arginine limitation and NO deficiency after the EAR. A first mechanism that may be of particular importance is increased utilization of the substrate for cNOS by the enzyme arginase, which hydrolyzes L-arginine into L-ornithine and urea (Chapter 2). It has previously been demonstrated that endogenous arginase activity is involved in the regulation of basal airway responsiveness to methacholine by attenuation of agonist-induced NO production, presumably by limiting the availability of L-arginine to cNOS [12]. We now demonstrated that arginase activity in tracheal homogenates obtained from allergen challenged guinea pigs is markedly increased after the EAR (Chapter 2). This increased arginase activity, which may be induced by Th2 cytokines [13-16] is importantly involved in the NO deficiency and subsequent AHR after the EAR, as incubation of perfused tracheal preparations with the specific arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA) completely normalized the AHR, whereas coincubation of this arginase inhibitor with the
nonselective NOS inhibitor N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME) reversed the effect of nor-NOHA. This clearly demonstrates that inhibition of arginase by nor-NOHA normalizes the airway responsiveness by restoring the production of cNOS-derived NO.

A second mechanism underlying the reduced L-arginine bioavailability after the allergen-induced EAR is inhibition of cellular uptake of the amino acid by endogenous (presumably eosinophil-derived) polycations (Chapter 3). In most cells, the L-arginine concentration is under important control of uptake via specific cationic amino acid transporters (CATs) of the y\textsuperscript{+} system [17]. In rat alveolar macrophages and tracheal epithelial cells it has been demonstrated that polycations, like major basic protein (MBP) as well as its analogue poly-L-arginine, can inhibit L-arginine uptake via inhibition of CATs, thereby decreasing NO synthesis by these cells [18]. The functional relevance of these observations for the regulation of airway responsiveness was indicated by the finding that poly-L-arginine causes AHR in perfused guinea pig tracheal preparations by inducing a deficiency of cNOS-derived NO [19]. The above mentioned effects of polycations on L-arginine transport, NO synthesis and airway responsiveness could be antagonized by the polyanion heparin [18,19]. In Chapter 3 we showed that while heparin did not affect airway responsiveness to methacholine in perfused tracheal preparation of unchallenged guinea pigs, the AHR after the allergen-induced EAR was completely normalized after incubation with the polyanion. Coincubation with L-NAME reversed the effect of heparin, indicating that heparin normalized the AHR by restoring the production of bronchodilating cNOS-derived NO. The potential involvement of eosinophil-derived polycations was supported by the observation that eosinophil peroxidase (EPO) release by bronchoalveolar lavage (BAL) eosinophils was 5-fold increased after the EAR. These findings could explain previous observations that inhaled unfractionated or low-molecular-weight heparins inhibit allergen-induced asthmatic reactions in allergic sheep [20,21], guinea pigs [22] and asthmatic patients [23-25]. Moreover, restoration of NO production may also explain the inhibitory effect of heparin on AHR to various contractile agonists in asthmatics [26,27] and in allergen-challenged sheep [20,28] and guinea pigs [22] \textit{in vivo}.

In conclusion, at least two mechanisms may underly the observed L-arginine limitation and subsequent NO deficiency and AHR after the EAR: (1) increased utilization of the NOS substrate by enhanced arginase activity and (2) reduced cellular uptake of the amino acid due to inhibition of CATs by polycations released during the inflammatory reaction. Remarkably, full restoration of the production of bronchodilating cNOS-derived NO could be obtained both by eliminating the substrate competition by using an arginase inhibitor, and by increasing the L-arginine availability by restoring the L-arginine uptake. Since increasing the L-arginine availability by restoring the uptake normalizes the AHR, it follows that arginase activity is becoming increasingly important in regulating the substrate availability for cNOS under conditions of (polycation-induced) substrate limitation.
Role of arginase and L-arginine homeostasis in peroxynitrite formation and development of airway hyperresponsiveness after the allergen-induced late asthmatic reaction

While a deficiency of cNOS-derived NO contributes to the development of AHR after the EAR [4], the AHR after the late asthmatic reaction (LAR) involves the formation of peroxynitrite, the proinflammatory and procontractile reaction product of NO and superoxide anion [29]. iNOS, which produces high concentrations of NO and is induced in human [30] as well as in guinea pig [7,31] airways during the allergen-induced LAR, may be important in the formation of peroxynitrite as indicated by the observation that increased levels of exhaled NO correlate with increased nitrotyrosine staining – a marker for peroxynitrite – in exhaled breath condensate of mild asthmatics [32]. Furthermore, increased nitrotyrosine staining in the airway epithelium and inflammatory cells in bronchial biopsies of asthmatic patients also correlates with iNOS expression, AHR and airway inflammation [33].

In Chapter 4 we demonstrated that reduced L-arginine availability to iNOS may be involved in the formation of peroxynitrite and subsequent AHR after the LAR. A relationship between reduced L-arginine availability to iNOS and peroxynitrite formation has initially been observed in macrophages by Xia et al. [34,35]. Particularly at low L-arginine concentrations, iNOS produces both NO by the oxygenase moiety of the enzyme and superoxide anion by its reductase moiety, leading to a rapid and highly efficient formation of peroxynitrite [34,35]. Increasing the concentration of L-arginine stimulates the production of NO, while the formation of superoxide anion, and hence peroxynitrite, is reduced [34].

Like after the EAR, L-arginine limitation to NOS after the LAR is caused by increased arginase activity and by reduced cellular L-arginine uptake induced by endogenous polycations. Thus, inhibition of arginase activity by nor-NOHA as well as scavenging polycations with the polyanion heparin normalized the AHR in perfused guinea pig tracheal preparations obtained after the LAR. The effects of nor-NOHA and heparin were completely reversed after coincubation with L-NAME, indicating that both agents normalize the AHR by restoring the production of bronchodilating NO. Thus, it appears that the AHR after the LAR is not caused by an excess of iNOS-derived NO, but rather by its deficiency, due to increased superoxide anion production by the enzyme and subsequent formation of peroxynitrite. In line with the pharmacological data, arginase activity was significantly increased in tracheal as well as in BAL cell homogenates obtained after the allergen-induced LAR. In addition, both the number and the activity of BAL eosinophils were markedly increased after the LAR.

Since increased arginase activity after allergen challenge results in an increased production of L-ornithine, we also studied the effect of L-ornithine on airway responsiveness, using perfused tracheal preparations from normal guinea pigs.
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(Chapter 5). In rat lung macrophages it has been reported that L-ornithine may inhibit L-arginine uptake via the \( \gamma^+ \)-system in a dose-dependent manner [36]. In line with these observations, we found that L-ornithine increased the airway responsiveness to methacholine by inducing a deficiency of cNOS-derived NO. In support of the importance of both arginase and L-arginine uptake in regulating the substrate availability to NOS, inhibition of arginase decreased the L-ornithine-induced AHR via increased NO production. This is fully in line with the previously described results obtained after the allergen-induced EAR and LAR, demonstrating that the AHR due to L-arginine deficiency and reduced production of bronchodilating NO could be normalized either by inhibition of arginase-induced substrate competition or by restoring cellular L-arginine uptake by scavenging endogenous polycations.

Furthermore, it was demonstrated that L-ornithine competitively inhibits arginase activity in lung, liver and kidney homogenates, indicating a feedback mechanism. Thus, L-ornithine can regulate arginase activity and NO homeostasis in two distinct ways: first by decreasing the L-arginine availability via inhibition of cellular L-arginine uptake and secondly by direct inhibition of arginase activity. Since in perfused guinea pig tracheal preparations the net effect of L-ornithine is increased airway responsiveness due to NO deficiency, it can be concluded that the impact of the inhibitory effect on L-arginine uptake is larger than that on arginase activity. The results described in this chapter also indicate that in addition to its direct competition with cNOS for the common substrate, L-arginine, arginase may further aggravate NO deficiency and AHR through the production of L-ornithine, presumably by inhibition of L-arginine uptake by \( \gamma^+ \) carriers.

Although studies on the role of arginase in airway responsiveness and asthma have thus far mainly focussed on substrate competition with NOS, a role for the arginase product L-ornithine and further downstream products like polyamines and L-proline can be envisaged. Polyamines and proline are known to be involved in cell proliferation and collagen production, respectively, which could play a role in airway remodelling in chronic asthma [14,37-40]. Indeed, increased levels of polyamines have been observed in mouse lung after allergen challenge [15] and in serum of asthmatic patients [41], respectively. Intriguingly, recent evidence suggests that growth factors, like EGF and PDGF, may be involved in the induction of arginase and of enzymes of the polyamine synthetic pathway [42-44]. Clearly, the role of arginase in airway remodeling needs further investigation.

**L-Arginine homeostasis and iNANC nerve-derived NO**

The iNANC nervous system is a most effective bronchodilating neural pathway of the airways. NOS inhibitors markedly reduce the iNANC relaxation of both guinea pigs [45-47] and human airways [48,49], indicating that NO is a major neurotransmitter of
the iNANC system. In Chapters 6 - 8 we studied the role of L-arginine homeostasis in regulating iNANC nerve-mediated, nNOS-derived NO production under basal conditions as well as after the allergen-induced EAR. To this aim, electrical field stimulation (EFS; 150 mA, 4 ms, 4s, 0.5 – 16 Hz)-induced relaxation was measured in precontracted tracheal open ring preparations of unchallenged and allergen challenged guinea pigs. In Chapter 6 we demonstrated that endogenous arginase activity is functionally involved in iNANC nerve activity in the airways, by attenuating the generation of nNOS-derived NO. Thus, nor-NOHA increased EFS-induced airway smooth muscle relaxation, particularly at the lower frequencies, i.e. the frequencies most sensitive to NOS inhibition. Coincubation with the NOS inhibitor Nω-nitro-L-arginine (L-NNA) reversed the effect of nor-NOHA to the level of L-NNA alone, indicating that arginase inhibition increases airway smooth muscle relaxation via increased NO generation. Since exogenous L-arginine mimicked the effect of nor-NOHA, it can be postulated that arginase regulates iNANC nerve-mediated NO generation via substrate competition with nNOS.

Interestingly, in Chapter 7 we showed that also iNANC relaxation is impaired after the allergen-induced EAR, due to deficiency of nNOS-derived NO. Thus, iNANC relaxation was markedly reduced after allergen challenge, to the level of unchallenged controls treated with L-NNA. In addition, L-NNA did not affect the impaired relaxation after allergen challenge, clearly indicating that NO deficiency is involved. Since incubation with exogenous L-arginine reversed the allergen-induced impaired relaxation to the normoreactive level of unchallenged controls, it can be concluded that limitation of L-arginine to nNOS underlies the NO deficiency. As already described in Chapter 2, arginase activity is increased after the allergen-induced EAR. Therefore, we studied the effect of nor-NOHA on iNANC nerve-mediated airway smooth muscle relaxations after allergen challenge. Nor-NOHA fully normalized the impaired relaxation at the lower, NO-sensitive, frequencies, which could be prevented by additional administration of the NOS inhibitor L-NNA. Taken together, these findings show that increased arginase activity after allergen challenge causes impaired iNANC nerve-mediated airway smooth muscle relaxation by attenuating the availability of L-arginine to nNOS.

In most cells, L-arginine requirements are met primarily by uptake of extracellular L-arginine via CATs. In some NO producing cells, L-arginine may also be generated by recycling from L-citrulline, the co-product of NO biosynthesis catalyzed by NOS, presumably to maintain NO production [50]. The conversion of L-citrulline to L-arginine is a two-step reaction involving argininosuccinate synthase (ASS), which converts L-citrulline and L-aspartate into L-argininosuccinate, and argininosuccinate lyase (ASL), which hydrolyses L-argininosuccinate into L-arginine and fumarate [50-52]. In some tissues it has been shown that the L-citrulline/L-arginine cycle is involved in iNANC nerve-mediated smooth muscle relaxation, particularly at relatively low L-arginine availability to nNOS induced by competitive inhibitors of the
enzyme [53-55]. In support, colocalization of ASS and ASL with nNOS has been reported [55-57].

In **Chapter 8** we investigated whether the L-citrulline/L-arginine cycle is active in iNANC nerves in guinea pig airways and whether the observed L-arginine limitation and subsequent NO deficiency after the EAR may involve impaired L-citrulline recycling. EFS-induced iNANC relaxation of tracheal preparations of unchallenged guinea pigs were not affected by treatment with exogenous L-citrulline and/or inhibition of ASS and ASL. Thus, L-citrulline recycling does not appear to be importantly involved in the regulation of basal iNANC nerve activity. By contrast, L-citrulline fully reversed the inhibitory effect of a submaximal concentration of L-NNA, particularly at the lower stimulation frequencies, which was prevented by inhibitors of ASS and ASL. These findings indicate that the L-citrulline/L-arginine cycle is operative in airway iNANC neurones and comes into play under conditions of low L-arginine utilization by nNOS.

As shown in Chapter 7, a deficiency of L-arginine underlies the reduced iNANC relaxation induced by nNOS after allergen challenge. Interestingly, incubation with exogenous L-citrulline fully normalized the impaired iNANC relaxation after the allergen-induced EAR (**Chapter 8**), similar to exogenous L-arginine. The full reversal by L-citrulline of the impaired iNANC response suggests that the L-arginine deficiency does not involve an enzymatic dysfunction in the citrulline/arginine cycle.

Taken together, the L-citrulline/L-arginine cycle is operative in guinea pig iNANC nerves and may be effective under conditions of low L-arginine availability to nNOS, e.g. after allergen challenge or in the presence of NOS inhibitors, presumably as a rescue mechanism to maintain cellular function. Recycling of L-citrulline to L-arginine does not appear to play a role under basal conditions, however. Moreover, enzymatic dysfunction in the L-citrulline/L-arginine cycle does not appear to be involved in the L-arginine limitation after the EAR.

**In vivo effects of arginase inhibition and of L-arginine on allergen-induced airway hyperresponsiveness**

Having established the importance of arginase in limiting L-arginine availability to NOS in allergic asthma *ex vivo*, we investigated the effects of the specific arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) and of L-arginine on the AHR after the EAR and LAR *in vivo* (**Chapter 9**). Using our guinea pig model of allergic asthma, we demonstrated that inhalation of ABH (15 min, 25 mM nebulizer concentration) acutely reversed the allergen-induced AHR after both the EAR and LAR. This effect was mimicked by inhalation of L-arginine (15 min, 1 M nebulizer concentration). As expected, inhalation of saline or the inactive enantiomer D-arginine did not affect the AHR after the allergen-induced EAR and LAR. Furthermore, inhalation of ABH 0.5 h before and 8 h after allergen challenge caused
significant protection against the AHR after both the EAR, while the AHR after the LAR was almost abolished. Remarkably, the dose of allergen needed to induce airway obstruction was significantly higher after pretreatment with ABH, demonstrating a protective effect of arginase inhibition on allergen-induced airway obstruction. This study for the first time demonstrates the importance of arginase in the pathophysiology of allergic asthma in vivo and indicates that arginase inhibitors may have therapeutic potential in this disease.

The importance of arginase in allergic asthma is supported by several studies in animal models of allergic asthma as well as in patients. Thus, pulmonary arginase activity was increased after allergen challenge in different mouse models [15,58,59]. Moreover, by microarray analysis of gene expression it was shown that genes related to L-arginine metabolism, including arginase I and arginase II, belonged to the most prominently overexpressed genes after allergen challenge [15]. Increased arginase activity after allergen challenge may be induced by Th2 cytokines, such as IL-4 and IL-13, known to be involved in allergic airway inflammation [14,15]. Arginase has been observed in the airway epithelium and in inflammatory cells, especially in (alveolar) macrophages and neutrophils [15,60,61].

Recent findings in asthmatic patients support the significance of arginase in the pathophysiology of allergic asthma. Thus, it was reported that protein expression of arginase I is increased in BAL cells of asthmatics [15]. In addition, enhanced mRNA expression of arginase I was observed in the airway epithelium and in inflammatory cells in bronchial biopsies of these patients [15]. Remarkably, a relationship between increased serum arginase activity and decreased plasma levels of L-arginine has been observed in asthmatic patients during exacerbation [62], supporting the role of arginase in regulating L-arginine availability. This finding further suggests that increased arginase activity and reduced L-arginine availability are not confined to the lung and that reduced levels of circulating L-arginine could contribute to NO deficiency and hyperresponsiveness observed in the airways. Interestingly, single nucleotide polymorphisms (SNPs) in arginase I and arginase II have recently been found to be associated with atopy and risk of childhood asthma [63].

In conclusion, the results presented in this thesis indicate a key role for arginase in the pathophysiology of allergic asthma and that arginase inhibitors may have therapeutic potential in this disease (Figure 1).
Arginase: a novel key enzyme in the pathophysiology of allergic asthma

Figure 1: Postulated role of arginase in the pathophysiology of allergic asthma. After the allergen-induced EAR, increased arginase activity contributes to the NO deficiency and subsequent AHR by limiting the L-arginine availability to cNOS. Increased arginase activity after the LAR attenuates the L-arginine availability to iNOS, which produces both NO and superoxide anion under these conditions, leading to the formation of procontractile and proinflammatory peroxynitrite and subsequent AHR. Since arginase activity is also increased in chronic allergic asthma, a role for arginase in airway remodeling induced by the production of the polyamines putrescine, spermidine and spermine as well as the collagen precursor L-proline from L-ornithine can be postulated. L-Ornithine also affects airway responsiveness by inhibiting L-arginine uptake, which might contribute to allergen-induced AHR by causing a deficiency of NO. As a breaking mechanism, L-ornithine may also cause feedback inhibition of arginase activity. Inhibition of arginase by nor-NOHA or ABH, as well as administration of exogenous L-arginine or L-citrulline may reverse arginase-induced effects after single allergen
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challenge by increasing the substrate availability to cNOS and iNOS. However, increasing the L-arginine availability in the absence of an arginase inhibitor could be detrimental in chronic asthma due to feeding the airway remodeling. ABH = 2(S)-amino-boronohexanoic acid, AHR = airway hyperresponsiveness, ASL = argininosuccinate lyase, ASS = argininosuccinate synthase, cNOS = constitutive nitric oxide synthase, iNOS = inducible nitric oxide synthase, NO = nitric oxide, nor-NOHA = N\textsubscript{ω}-hydroxy-nor-L-arginine, O\textsubscript{2}\textsuperscript{-} = superoxide anion, OAT = ornithine aminotransferase, ODC = ornithine decarboxylase, ONOO\textsuperscript{-} = peroxynitrite, P5C = pyrroline-5-carboxylate.

Arginase and aging

In Chapter 10 we investigated the role of arginase in age-induced alterations in airway function. Previously, it has been demonstrated that vascular aging, characterized by reduced endothelium-dependent vascular smooth muscle relaxation, is associated with the formation of peroxynitrite [64]. Thus, although eNOS expression and activity are upregulated, levels of NO are markedly reduced, while the superoxide anion generation and staining for 3-nitrotyrosine are increased in aged animals [64]. In the airways, formation of peroxynitrite is importantly involved in the development of AHR 24 h after single allergen challenge [29]. As indicated in Chapter 4, increased arginase activity is involved in the peroxynitrite formation after allergen challenge by limiting the L-arginine availability to iNOS, which produces both NO and superoxide under this condition [34,35]. Interestingly, arginase activity has been found increased with age in rat aortic rings and rabbit cavernous tissue, leading to reduced vascular function [65,66].

A positive relationship between age and increased airway responsiveness has been reported [67]; however, the underlying mechanisms are currently unknown. In Chapter 10 we studied the effect of age on airway responsiveness in young adult (3-5 months), mature (7-11 months) and senescent (20-23 months) guinea pigs. Airway responsiveness was increased in mature and senescent animals as compared to the young adults both in vivo and in perfused tracheal preparations ex vivo. In perfused preparations obtained from mature and senescent animals, both SOD and L-NAME normalized the age-related AHR, indicating a prominent role for peroxynitrite in this process. Importantly, arginase activity in the airways of mature guinea pigs was markedly increased as compared to the young animals. The importance of arginase was supported by the finding that nor-NOHA normalized the AHR to normoresponsiveness seen in young adults. Coincubation with L-NAME reversed the effect of nor-NOHA, confirming that arginase inhibition normalizes the airway responsiveness by restoring the production of bronchodilating NO. Moreover, the age-related AHR was decreased by incubation with exogenous L-arginine. Based on these findings, we postulate that increased arginase activity in the aging airway contributes to the development of age-related AHR by limiting the availability of L-
arginine to NOS, which then produces both NO and superoxide anions, leading to the formation of peroxynitrite.

Main conclusions

In conclusion, the studies described in this thesis have revealed that:

- Arginase activity in the airways is increased after allergen challenge, both after the EAR (Chapter 2) and the LAR (Chapter 4).
- Increased arginase activity underlies the deficiency of cNOS-derived NO and subsequent AHR after the EAR (Chapter 2). In addition, release of (eosinophil-derived) polycations may contribute to the observed NO deficiency after the EAR, presumably via inhibition of cellular L-arginine uptake by specific CAT transporters (Chapter 3).
- Increased arginase activity as well as release of endogenous polycations contribute to the allergen-induced AHR after the LAR by limiting the substrate availability to iNOS. L-Arginine limitation to iNOS favors the synthesis of both NO and superoxide anion by this enzyme, leading to the formation of the procontractile and proinflammatory nitrogen species peroxynitrite (Chapter 4).
- The arginase product L-ornithine causes AHR by inducing a deficiency of cNOS-derived NO, presumably by inhibition of cellular L-arginine uptake. In addition, L-ornithine negatively regulates arginase activity via competitive inhibition of the enzyme (Chapter 5).
- Constitutive arginase activity attenuates the iNANC nerve-mediated NO production and airway smooth muscle relaxation under basal conditions by limiting the availability of L-arginine to nNOS (Chapter 6).
- Increased arginase activity after the allergen-induced EAR leads to impaired iNANC nerve-mediated airway smooth muscle relaxation by inducing a deficiency of nNOS-derived NO (Chapter 7).
- Recycling of L-citrulline to L-arginine is involved in the regulation of iNANC nerve-mediated NO production and airway smooth muscle relaxation under conditions of low L-arginine availability. However, putative dysfunction of the L-citrulline/L-arginine cycle does not underly the L-arginine limitation after the EAR (Chapter 8).
- Inhalation of the arginase inhibitor ABH acutely reverses the allergen-induced AHR after the EAR and LAR in vivo, which can be mimicked by inhalation of L-arginine. In addition, pretreatment with ABH considerably reduces the sensitivity of the airways to inhaled allergen and protects...
against the development of allergen-induced AHR after both after the EAR and the LAR. Therefore, arginase inhibitors may have therapeutic potential in allergic asthma (Chapter 9).

- During aging, airway hyperresponsiveness develops due to reduced formation of bronchodilating NO and increased formation of peroxynitrite. Reduced L-arginine availability to NOS caused by increased arginase activity underlies this age-related AHR (Chapter 10).

References
Summary and conclusions


